2337-Pos Board B323
Structural and Functional Analyses for the Interaction Between Inward Rectifier K^+ channels and Cations
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The gate of ion channels is a physical constraint for the ion permeation at the membrane-embedded domain, and a target for various physiological stimuli. The inward rectifier K^+ (Kir) channels possess unique cytoplasmic domain that contributes to a half of the ion conduction pathway. Whereas the domain is known to behave as a hub domain for the channel activators to control the activation gating at the transmembrane domain, it remains unclear whether the extra-membrane domain itself functions as the gate. By detecting the distribution of an anomalous signal of the cations in the crystal structure of the cytoplasmic domain of Kir channel subunit Kir3.2, we qualitatively analyzed the interaction between various cations and the cytoplasmic pore. The multi-valent cations non-selectively interact with the site caged by Glu236 and Met313 at the cytoplasmic pore. Mg^{2+}, but not spermine, competitively binds to the same site and prevents the association of monovalent cations. The substituted cysteine accessibility method was employed to analyze the interaction of Mg^{2+} and the cytoplasmic pore of Kir3.2. When the channel is in a closed state, Mg^{2+} selectively blocks the modification of Met313 by intracellular sulfydryl modifier, methanethiosulfonate-ethyltrimethylammonium. These results indicate that the Kir3.2 crystal structure associates with the closed state and interacts with cations dependent on the valence of positively charged moiety, thereby suggesting that the cytoplasmic domain functions as a gate by itself by altering the inner diameter of the pore.

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Activation of Kir4.2 by Extracellular Potassium
Johan M. Edvinsson, Anish J. Shah, Lawrence G. Palmer.
The inwardly rectifying K^+-channel, Kir4.2, is regulated by external K^+, a property that it shares with at least two other members of the Kir-family (Kir1.1 and 4.1). This regulation is marked as a slow increase (~10s of minutes) of the whole-cell currents when $[K^+]_o$ is increased. Here we investigate the mechanism of K^+_o-dependent regulation of Kir4.2. The K^+_o-dependent regulation is specific to the homomeric form of the channel and is abolished when Kir4.2 is coexpressed with Kir5.1. Unlike Kir1.1, there is no coupling between the sensitivity to intracellular pH and K^+_o-dependent regulation of Kir4.2. Furthermore, the rate of activation is slower then the rate of inactivation (~30 min vs. ~ 2 min) and there is an inverse relationship between the rate of activation and the $[K^+]_o$. Using biotinylation we show that K^+_o-dependent regulation does not involve a change in the surface expression of the channel, hence K^+_o affects the properties of the channel at the cell surface. Patch clamp measurements show that there is no difference in the open probability of Kir4.2 in high or low K^+-bath solutions (P_o~0.7). Using a kinetic model we show that these findings are compatible with the channel existing in at least three states at the plasma membrane, one long-lived, K^+_o-insensitive, inactivate state, one unstable intermediate state, and one active state. K^+_o affects the rate of transition from the intermediate to the active state, and the measured open probability (~0.7) is a property of the active state.

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Modulation of Kir1.1 Inactivation by External Divalents
Henry Sackin, Mikheil Nanazashvili, Hui Li, Lawrence G. Palmer, Lei Yang.
Kir1.1 inactivation, associated with transient internal acidification, was strongly dependent on external K, Ca and Mg. In 1mM K, a 15 min transient acidification (internal pH 8 to pH 6.3) produced 91 ± 2% inactivation in 2mM Ca, but only 18 ± 4% inactivation in the absence of external Ca and Mg. In 100mM external K, the same acidification protocol produced 29 ± 5% inactivation in 10mM external Ca but no inactivation when extracellular Ca was reduced below 2mM (with 0 Mg). External Ca was more effective than external Mg at producing inactivation, but Mg caused a greater degree of open channel block than Ca, making it unlikely that Kir1.1 inactivation arises from divalent block. Since the Ca sensitivity of inactivation persisted in 100mM external K, it is also unlikely that Ca enhances Kir1.1 inactivation by reducing the local K concentration at the outer mouth of the channel. Removal of 4 surface, negative side-chains at E92, D97, E104, E132 (Kir1.1b) increased the sensitivity of inactivation to external Ca (and Mg); whereas adding a negative surface charge (N105E-Kir1.1b) decreased the sensitivity of inactivation to Ca and Mg. This result is consistent with negative surface charges stabilizing external K in the selectivity filter or at the S_0-K binding site just outside the filter. In this model, extracellular Ca and Mg potentiate the slow, K-dependent, inactivation of Kir1.1 by allosterically destabilizing the conducting conformation of the selectivity filter, independent of divalent block in the permeation pathway.

2340-Pos Board B326
Properties and Molecular Composition of Mouse and Canine Cardiac Purkinje Fiber ATP-Sensitive K^+ Channels
Li Bao, Eirini Kefalogianni, Minyoun Hong, Alexander Coetzee, Gregory Morley, Glenn Fishman, William Coetzee.
ATP-sensitive potassium (K_{ATP}) channels consist of pore-forming (Kir6.1 and Kir6.2) and sulfonylurea receptor (SUR1, SUR2A or SUR2B) subunits. We compared the biophysical and pharmacological properties, as well as the molecular composition of K_{ATP} channels in cardiac Purkinje cells and ventricular myocytes. Mouse Purkinje cells and myocytes were enzymatically isolated from Cntn2-EGFP Purkinje cell reporter mice. K_{ATP} channels recorded from Purkinje cells (inside-out patch clamp configuration) were inhibited by ATP with an IC_{50} of 125 ± 18μM (Hill coefficient of 2.6), which was significantly higher than that of the ventricular K_{ATP} channel (68 ± 10μM). The slope conductance of the Purkinje cell K_{ATP} channel in symmetric 140 mM K^+ was also lower (57 ± 3pS versus 76 ± 1pS in the ventricle). Kinetic analysis showed similar mean open (respectively 2.2 ± 0.16 and 2.9 ± 0.95ms) and closed times (respectively 0.4 ± 0.02 and 0.4 ± 0.02ms) in Purkinje cells and ventricular myocytes. The Purkinje fiber K_{ATP} channel was activated preferentially by tolbultamide (200μM) and less by levcromakalim (30μM), whereas the reverse pharmacological profile was observed for the ventricular K_{ATP} channel. Using real-time semi-quantitative RT-PCR, we detected expression of Kir6.1, Kir6.2, and SUR2 mRNA in both mouse cell types. Purkinje fibers expressed more Kir6.1 and less total SUR2 than ventricular myocytes. Conventional RT-PCR demonstrated increased SUR2B expression relative to SUR2A in Purkinje cells. Western blotting confirmed expression of Kir6.1, Kir6.2, SUR2A and SUR2B protein in both dog Purkinje fibers and ventricular tissues with Kir6.1 and SUR2B levels elevated in the Purkinje fiber. Consistently, our confocal microscopy imaging data demonstrated the presence of Kir6.1, Kir6.2, and SUR2B subunits on the Purkinje cell. In summary, K_{ATP} channels in cardiac Purkinje cells manifest unique channel properties and molecular composition. They may have a differential electrophysiological response (eg. conduction velocity and arrhythmia) during periods of ischemia.

2341-Pos Board B327
Sulphonylurea Receptors Regulate Kir6.2 Subunits Allosterically via a Salt Bridge in Cardiac K_{ATP} Channels
Hussein N. Rubaiy, Richard D. Rainbow, Dave Lodwick, Robert I. Norman.
ATP sensitive potassium (K_{ATP}) channels play important roles in many tissues, coupling metabolic status to membrane potential. In heart, they comprise subunit hetero-octamers of $Kir6.2_4/SUR2A_4$.
A minimal sequence (residues 1294-1358) in nucleotide binding domain-2 of SUR2A has been indentified previously by us to bind Kir6.2 (Rainbow et. al. Biochem. J. 2004;379:173-181). Co-immunoprecipitation of chimaeras of Kir6.2/Kir2.1 with maltose binding protein (MBP)-tagged SUR2A(1294-1358) fragments identified the cognate binding domain in Kir6.2 (residues 315-390). Mutagenesis of charged residues in Kir6.2, D323K and K338E, conserved in Kir6.1 but not Kir2.1, was alone sufficient to significantly reduce co-immunoprecipitation of MBP-SUR2A(1294-1358) by ~50 % ($P<0.0009$) and ~80% ($P<0.0001$), respectively. Similarly, co-immunoprecipitation with Kir6.2 of chimaeras of SUR2A(1294-1358) containing multi-drug resistance protein-1 (MRP1) sequence further refined the SUR2A binding motif to residues 1318-1337. Mutagenesis of charged residues in SUR2A, E1318R, K1322D and Q1336E, conserved in SUR1 but not MRP1, reduced co-immunoprecipitation.
Whole cell patch clamp of Kir6.2(K338E)/SUR2A WT channels 48-72 h after transfection in HEK 293 cells revealed increased sensitivity to agonist, pinacidil (EC_{50} = 4.5 0.3 versus 39.6 13 μM with Kir6.2-WT), and reduced sensitivity to antagonist, glibenclamide (IC_{50} = 103 28 versus 3.1 0.8 nM with Kir6.2-WT). Expression of the double mutant Kir6.2(K338E)/SUR2A(E1318R) restored wild-type properties (EC_{50} pinacidil = 46.7 4.9 μM; IC_{50} glibenclamide = 6.4 0.9 nM). Similar results were observed for the equivalent residue on Kir6.1 (R347). Furthermore, inside-out patch clamp revealed that the IC_{50} for ATP was unaffected in Kir6.2K338E/SUR2A channels (27.5 ± 2.0 versus 23.8 ± 1.7 M with Kir6.2-WT, $P>0.5$).
Together, these data provide evidence for the transmission of allosteric information via a salt bridge between SUR2AE1318 and Kir6.2K338 in cardiac K_{ATP} channels, while ATP-sensitivity remains unaltered.

2342-Pos Board B328
The Predominant Mechanisms of Pathogenic Hyperactivity of Mutant ABCC8/KCNJ11-Coded KATP Channels
Andrey P. Babenko, Martine Vaxillaire.
We discovered numerous congenital diabetes (CD), insulin release-inhibiting mutations in ABCC8/KCNJ11 genes coding for the pancreatic beta-cell (SUR1/Kir6.2)4 KATP channel (Diabetes 53:2719; N Engl J Med 355:456; Diabetes 56:1737; J Biol Chem 283:8778; reviewed in Endocr Rev 29:265).